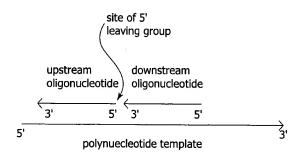
Fig. 1a

Fig. 1b

Fig. 1c





H₃C
$$\stackrel{\text{H}}{\underset{\text{N}}{\bigcap}}$$
 $\stackrel{\text{H}}{\underset{\text{N}}{\bigcap}}$ $\stackrel{\text{H}}{\underset{\text{N}}$ $\stackrel{\text{H}}{\underset{\text{N}}{\bigcap}}$ $\stackrel{\text{H}}{\underset{\text{N}}{\longrightarrow}}$ $\stackrel{\text{H}}{\underset{\text{N}}{\longrightarrow}}$ $\stackrel{\text{H}}{\underset{\text{N}}{\longrightarrow}}$ $\stackrel{\text{H}}{\underset{\text{N}}{\longrightarrow}}$ $\stackrel{\text{H}}{\underset{\text{N}}{\longrightarrow}}$ $\stackrel{\text{H}}{\underset{\text{N}}{\longrightarrow}}$ $\stackrel{\text{H}}{\underset{\text{N}}{\longrightarrow}}$ $\stackrel{\text{H}}{\underset{\text{N}}}$

ligation of duplex DNA ligation of ssDNA (SEQ ID NO:15) (SEQ ID NO:12) TGTACGCTGGATGCA GATCAGGT TTCATGCGACCT CTAGTCCAAAGTGCTCGG ACGTAGGTCGCATG T^T (SEQ ID NO:13) (SEO ID NO:15) $_{\mathrm{T}}$ TGTACGCTGGATGCA $_{\mathrm{T}}$ TCCAGCGTACT $_{\mathrm{T}}$ GATCAGGT,TTCACGAGCCTG ${f T}_{f T}{f CATGCGACCT_s}{f ACGTAGGTCGCATGT}^{f T}$ (SEQ ID NO:14) (SEQ ID NO:16) one pot ligation / cyclization of ssDNA (SEQ ID NO:17) GTTTTATACAAAACCTGGCA T(SEQ ID NO:19) (SEQ ID NO:18) AGTGATCAAGTCCTCTGA (SEQ ID NO:20) $_{
m T}$ TCAGCAAAATATGT $_{
m s}$ TTTGGACCGTTGGT $_{
m T}$ (SEQ ID NO:21)

| rxn.type | conversion | isolated yield |
|----------------------|-----------------------------------|----------------|
| ssDNA ligation | >90% | 44% |
| duplex ligation | 75% | 36% |
| ligation/cyclization | >90% (1st step) 50% (2nd step) | 20% |

 $^{\mathrm{T}}$ CTGCTTCACTAGT. TCAGGAGACTGTTCA $^{\mathrm{G}}$

Fig. 4a

Fig. 4b

exonuclease/hydrolysis susceptibility

5'-GATCAGGT_{Ps}TTCACGAGCCTG-3' (SEQ ID NO:14)

endonuclease susceptibility

(SEQ ID NO:16)

template for replication / transcription

(SEQ ID NO:22)

5'-TAATACGACTCACTATA 5'-TAATACGACTCACTATA 3'-ATTATGCTGAGTGATATCCTGCCTATTCCGAGCACTT_{P8}TGGACTAG (SEQ ID NO:23)

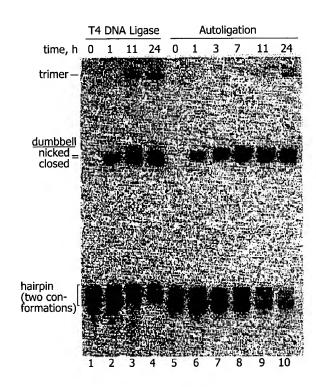


Fig. 6

Cas. 15.

linear probes

(SEQ ID NO:26)
5' 3'
GTGGGCGCCGPs.

(SEO ID NO:27) MUT target 3'CCACCACCGAGGCAGCCACACCCATTC5'

(SEQ ID NO:28) 5'MM target 3'CCACCACCCGAGGCCGCCACACCCATTC5'

(SEQ ID NO:29) 3'MM target 3'CCACCACCCGAGGAAGCCACACCCATTC5'

(SEQ ID NO:30) MMM target 3'CCACCACCCGAGGCAGGCACACCCATTC5

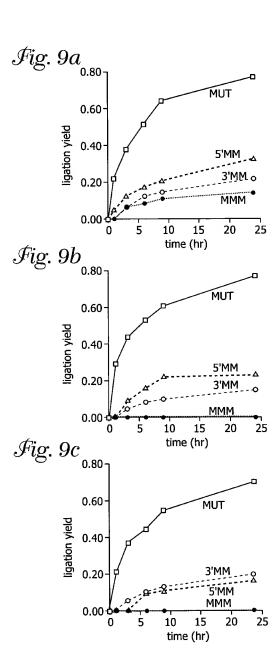
 $(\texttt{SEQ ID NO:31}) \ \, \underbrace{ \begin{array}{c} \texttt{cyclization probe} \\ \texttt{T} \end{array} }^{T} \underbrace{ \begin{array}{c} \texttt{ACATTAGCACTATAAGCAC} \\ \texttt{T} \\ \texttt{T} \\ \texttt{GTGGGCGCCGP}_{\texttt{B}} \end{array} }^{T} \underbrace{ \begin{array}{c} \texttt{T} \\ \texttt{T} \\ \texttt{T} \\ \texttt{CTGGTGTGGG} \end{array} }^{T} \\ \underbrace{ \begin{array}{c} \texttt{T} \\ \texttt{T}$

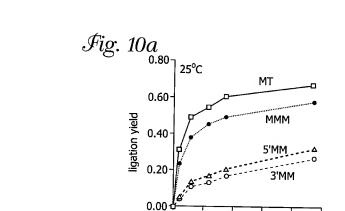
(SEQ ID NO:27) MUT target 3'CCACCCGAGGCAGCCACACCCATTC5'

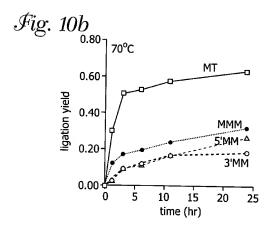
(SEQ ID NO:28) 5'MM target 3'CCACCACCCGAGGCCGCCACACCCATTC5'

(SEQ ID NO:29) 3'MM target 3'CCACCACCCGAGGAAGCCACACCCATTC5'

(SEQ ID NO:32) MMM target 3'CCACCACCGAGGCAGCCAAACCCATTCS







5

10

15

time (hr)

20

25

Azino.

 $(SEQ\ ID\ NO:31) \ \ \frac{cyclization\ probe}{GGCGCGCG_{Ps}} \ \ \frac{3^{1}}{T}TTTACATTAGCACTATAAG_{TC} \\ GGCGCCG_{Ps} \ \ \ TCGGTGTGGGTTTTC$

(SEQ ID NO:33) MUT target 3'ACTACGCGGCAGCCACACCCAAAAGTTC

(SEQ ID NO:34) 3'MM target 3'ACTACGCGGAAGCCACACCCAAAAGTTC5'

(SEQ ID NO:35) MMM target 3'ACTACGCTGCAGCCACACCCAAAAGTTC5

Fig. 11a

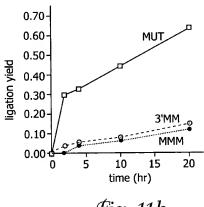


Fig. 11b

Fig. 12a

linear probes

(SEQ ID NO:36) TGAGAACGGGTGT-: PaGGCTGCC

(SEQ ID NO:37) WT target

5'GTCAGCGCACTCTTGCCCACACCGC CGCCCACCACCACCAGCTTATA31

(SEQ ID NO:38) MUT target 5'GTCAGCGCACTCTTGCCCACACCGACGG-

CGCCCACCACCAGCTTATA3'

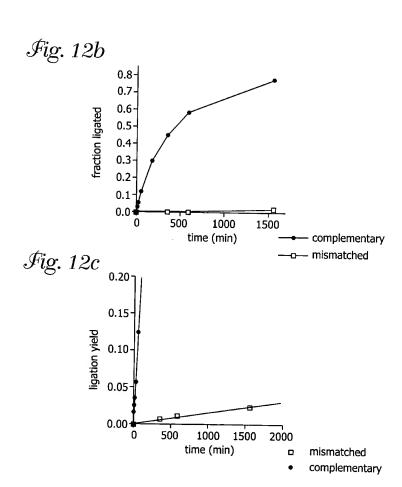
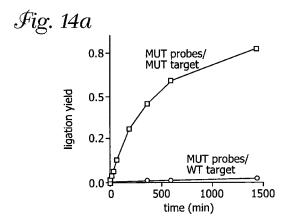
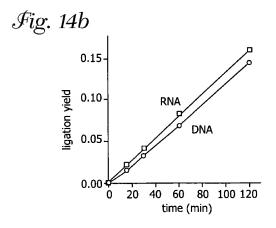


Fig. 13





| (5) | SIBCLASS | |
|-----|----------|-----|
| ٠. | · | ÷ ; |
| | .2 | i~- |

| temperature | [target] | fold turn | fold turnover | |
|-------------|----------|-------------------------|----------------------|--|
| (°C) | (nM) | no cycling ^a | cycling ^b | |
| 22 | 1 | 24 | | |
| | 10 | 1.6 | 1.0 | |
| | 100 | 1.0 | 1.0 | |
| 27 | 1 | 13 | 14 | |
| | 10 | 1.6 | 3.0 | |
| | 100 | 1.2 | 1.2 | |
| 32 | 1 | 40 | 51 | |
| | 10 | 4.6 | 4.7 | |
| | 100 | 2.3 | 2.3 | |
| 37 | 1 | 30 | 44 | |
| | 10 | 5.9 | 6.2 | |
| | 100 | 2.2 | 2.2 | |

 $^a\text{Simple}$ incubation of 10 μM probes with target for 24 hr followed by gel electrophoresis and quantitation of ligated product. b24 hr of thermal cycling (30 min at temp. shown, followed by 45 sec at 95°C).

Fig. 15a

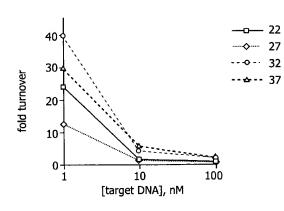
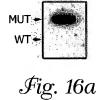
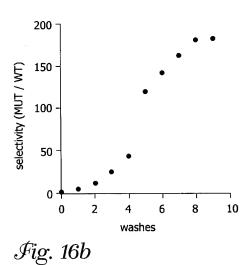


Fig. 15b







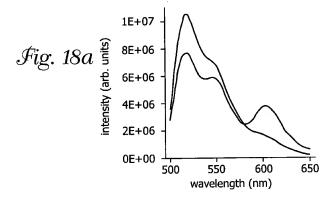


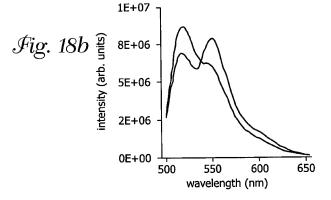
universal probe (FAM label)

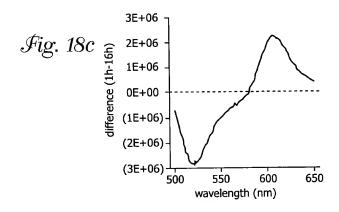
Fig. 17



DRAFTS







SECOLASS

Fig. 19

probes 3' (SEQ ID NO:36)

TGAGAACGGGTGT: -seGGCXGCC (X=G,T)

WT target

5'GTCAGCGCACTCTTGCCCACACCGCCGCCGCCCACCACCACCACCAGCTTATA3' (SEQ ID NO:37)

MUT target (SEQ ID NO:38)

MUT RNA target (SEQ ID NO:40)
5'GCGCACUCUUGCCCACACCGACGCGCCG'

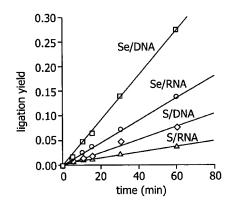


Fig. 20

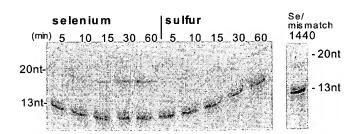


Fig. 21